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## **Chapter 20**

### **Nanowire Biosensors for Label-Free, Real-Time, Ultrasensitive Protein Detection**

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## Abstract

Sensitive and quantitative analysis of proteins is central to disease diagnosis, drug screening, and proteomic studies. Among recent research advances exploiting new nanomaterials for biomolecule analysis, silicon nanowires (SiNWs), which are configured as field-effect transistors (FETs), have emerged as one of the most promising and powerful platforms for label-free, real-time, and highly-sensitive electrical detection of proteins, as well as, many other biological species. Here, we describe a detailed protocol for realizing SiNW biosensors for protein detection that includes SiNW synthesis, FET device fabrication, surface receptor functionalization, and electrical sensing measurements. Moreover, incorporating both p-type and n-type SiNWs in the same sensor array provides a unique means of internal control for sensing signal verification.

**Key Words:** nanowire; chemical vapor deposition; FET; surface modification; cancer marker; isoelectric point.

## 1. Introduction

SiNWs possess unique structural and chemical characteristics to include: diameters similar to proteins; high surface-to-volume ratios; and chemically well-defined and tailorable silicon oxide surfaces(1-3). These characteristics have enabled SiNWs to be configured as high-performance FETs for real-time, sensitive detection of proteins and other biomolecules without the need of labeling(4-8). The fundamental principle of the nanowire electrical detection is the field effect(9-10), by which a small variation of charge or electric potential at the nanowire surface occurs during biomolecule binding that leads to a corresponding change of conducting charge carrier density (i.e., holes for p-type and electrons for n-type semiconductors) inside the nanowire in a manner similar to electrical field gating that turns a conventional FET on or

off(**11**). Functionalization of the nanowire surface with biomolecule receptors, such as monoclonal antibodies, enables real-time electrical measurement of specific target protein binding in solutions that are delivered to the sensor surface, while the subsequent delivery of the buffer leads to the detection of a specific protein unbinding and the generation of a nanowire sensor surface that is capable of additional analysis.

This article provides a detailed description of how to synthesize high-quality, single-crystal SiNWs by using a chemical vapor deposition (CVD) method to fabricate high-performance SiNW FETs, and then, to convert these SiNW FETs into protein sensors by surface modification with monoclonal antibodies, although modification with other receptors is also possible.

Proteomics research is elucidated and expected to continue for the search to discover biomarkers, when measured in parallel, with the potential to improve the diagnosis of disease(**12-13**). We exemplify the capabilities of SiNW sensors with the detection of prostate specific antigen (PSA) (**14**), an important and widely-used cancer marker, and then, show the multiplexed detection of three distinct cancer markers.

## **2. Materials and equipment**

### **2.1. Synthesis of semiconductor SiNWs by the CVD method**

1. Single crystal silicon wafer (Nova Electronic Materials, Flower Mound, TX): 3" N <100>, <0.005 ohm-cm, 356—406  $\mu\text{m}$  thick, SSP prime grade, flats & with 500 nm  $\pm$  5% thermal oxide
2. Poly-L-lysine (Ted Pella, Redding, CA): 0.1% w/v aqueous
3. Gold colloid (Ted Pella): 20 nm diameter,  $7.0 \times 10^{11}$  particles/ml
4. Oxygen plasma cleaner (PJ Benchtop, AST Products, Billerica, MA)
5. Optical microscope (Olympus, Tokyo, Japan)
6. Sonicator (Branson, Danbury, CT)

7. Homebuilt chemical vapor deposition (CVD) system, composed of the following parts (**15**):

- (1) Tube furnace (Lindberg Blue/M, New Columbia, PA)
- (2) Mass flow controllers (MKS Instrument, Andover, MA)
- (3) Dry pump (Kashiyama, Fremont, CA)
- (4) Capacitance pressure gauge: (MKS Instruments, Andover, MA)
- (5) Automated valve (MKS)
- (6) Electronic pressure controller (MKS)
- (7) Silane gas (99.9% SiH<sub>4</sub>, Voltaix, Branchburg, NJ), diborane (100 ppm B<sub>2</sub>H<sub>6</sub> in H<sub>2</sub>, Voltaix), phosphine (1000 ppm PH<sub>3</sub> in H<sub>2</sub>, Voltaix), argon (99%, Matheson Tri-gas, Montgomeryville, PA), hydrogen (99.9995%, Matheson Tri-gas)

## **2.2. Fabrication of SiNW-FET devices**

1. Lift-off resist (LOR) 3A (MicroChem, Newton, MA)
2. S1805 photoresist (MicroChem)
3. MF319 developer (MicroChem)
4. Remover PG (MicroChem)
5. Buffer hydrofluoric (HF) acid (Transene, Danvers, MA)
6. Nicket slug (Alfa Aesar, Ward Hill, MA): 99.995%, 3.175 mm diameter × 3.175 mm length
7. Spin coater (Headway Research, Garland, TX)
8. Hotplate (Fisher Scientific, Pittsburgh, PA)
9. Photolithography mask aligner (AB-M, Scotts Valley, CA)
10. Metal thermal evaporator (Sharon Vacuum, Brockton, MA)
11. Plasma-enhanced chemical vapor deposition (Nexx, Billerica, MA)
12. Rapid thermal processor (Applied Materials, Santa Clara, CA)

13. Probe station (Cascade, Beaverton, OR)
14. Lock-in amplifier (Stanford Research Systems, Sunnyvale, CA) DSP dual-phase
15. Current pre-amplifier (DL Instrument, Ithaca, NY)
16. Multifunctional DAC card and I/O board (National Instruments, Austin, TX)

### **2.3. Surface modification of SiNW biosensors with monoclonal antibody**

1. 3-(trimethoxysilyl)propyl aldehyde (90%, United Chemical Technologies, Bristol, PA)
2. Acrodisc syringe filter (Pall, Ann Arbor, MI): 0.2 micron cut-off, HT tuffryn membrane, non-pyrogenic
3. Polydimethylsiloxane (PDMS, Essex Brownwell, Atlanta, GA): made from Sylgard 184(**16**).  
The overall dimension of the PDMS is  $\sim 10 \times 10 \times 5$  mm (length  $\times$  width  $\times$  height), and the fluidic channel is  $5 \times 0.5 \times 0.05$  mm. The  $\sim 0.7$  mm diameter inlet and outlet holes are located at the two ends of the channel.
4. Polyethylene tubing (Becton Dickinson, Franklin Lakes, NJ): inner diameter 0.38 mm, outer diameter 1.09 mm
5. Ethanolamine (Sigma-Aldrich, Milwaukee, WI)
6. Antibody binding buffer: 10 mM phosphate buffer solution (containing 4 mM sodium cyanoborohydride), pH 8.4
7. Prostate specific antigen antibody (NeoMarkers, Fremont, CA): mouse monoclonal PSA antibody, without bovine serum albumin or azide
8. Syringe pump (Harvard Apparatus, Holliston, MA)

### **2.4. Protein detection by SiNW biosensors**

1. Sensing buffer: 10  $\mu$ M phosphate buffer solution, pH 8. Note that the pH of the buffer solution is selected depending on the isoelectric points (pIs) of different antigen analytes, based

on the requirement of maximizing antigen's surface charges without affecting its activity.

Generally a pH value of  $pI \pm 2$  is suggested for preparing the buffer.

2. Prostate specific antigen (PSA, EMD Bioscience, Gibbstown, NJ)

## **2.5. Multiplexed antibody functionalization and cancer marker detection by SiNW biosensors**

1. Carcinoembryonic antigen (CEA, EMD Bioscience, Gibbstown, NJ)
2. Anti-CEA (clone COL-1, Neomarkers, Fremont, CA)
3. Mucin-1 (EMD Bioscience, Gibbstown, NJ)
4. Anti-mucin-1 (clone B413, Abcam, Cambridge, MA)

## **3. Methods**

The synthesis and fabrication of SiNW electrical sensors involves three key steps: (i.) uniform, single-crystal p-type and n-type SiNWs are synthesized by a metal nanoparticle-catalyzed CVD method(*1,15*); (ii.) addressable SiNW electronic FET device arrays are fabricated by conventional photolithography, and subsequent silicon nitride ( $Si_3N_4$ ) passivation and thermal annealing are used to prevent the metal electrodes from degradation in electrolyte solution and increase the device conductivity(*15*); and (iii.) antibody receptors are covalently linked to SiNW surfaces to enable the capability of target recognition. After the SiNW sensor is prepared, solutions of protein analytes are delivered to the SiNW sensor surface via a microfluidic channel, and the binding/unbinding of proteins at the SiNW surface is recorded by the nanowire electrical conductance change. Furthermore, by incorporating both p-type and n-type SiNW FETs in a same sensor array, we are able to simultaneously measure the conductivity change in both SiNW devices that enables a unique means for the discrimination of false positive signals.

### **3.1. Synthesis of semiconductor SiNWs by the CVD method**

1. Use a diamond scribe to cut a silicon <100> wafer with 600 nm thermal oxide into small chips of desired size (e.g., 2 cm × 2 cm). These chips will be used as growth chips for SiNWs. Sonicate these chips in acetone and then in ethanol for 10 min each. Then, place one growth chip on a clean surface with the polished side facing up, and cover its surface with 200 µl poly-L-lysine (0.1% in deionized (DI) water) for 2 min. Afterwards, rinse the chip surface with DI water for 5-10 sec, and then dry the chip with a N<sub>2</sub> gas stream.
2. Cover the growth chip surface with 200 µl gold colloid solution: 20 nm colloid diameter, 1:4 v/v dilution in DI water for 10 sec. Rinse the chip surface with DI water for 5-10 sec, and then dry the chip with a N<sub>2</sub> gas stream (*see Note 1*).
3. Place the growth chip inside an oxygen plasma cleaner, and clean the chip surface using 100 W plasma power and 50 sccm (i.e., standard cubic centimeters per minute; 1 sccm = 1.7 × 10<sup>-8</sup> m<sup>3</sup>/s) O<sub>2</sub> flow, for 5 min.
4. Insert the growth chip in the middle of a quartz tube (inner diameter ~ 1 inch), and place the quartz tube in the tube furnace of a CVD system, with one end connected to reactant gas lines and mass flow controllers, and the other end connected to a control valve and a dry pump. A schematic of the CVD setup is shown (*see Fig. 1A*).
5. Close all the gas lines, and completely open the valve to fully evacuate the quartz tube to pressure less than 3 mTorr. Then start to flow 10 sccm Ar, and increase the tube furnace temperature to the designated temperature for SiNW growth.
6. At the growth temperature, start to flow all reactant gases and control the valve opening degree to achieve certain pressure inside the quartz tube for growing different designed nanowires. A typical recipe for p-type SiNWs is: 460°C, 10 sccm Ar, 6 sccm SiH<sub>4</sub>, 7.5 sccm B<sub>2</sub>H<sub>6</sub> (100 ppm in H<sub>2</sub>), total chamber pressure is 25 Torr. A typical p-type SiNW growth rate at



this condition is  $\sim 1.2\text{--}1.5\text{ }\mu\text{m}/\text{min}$ . A typical recipe for n-type SiNWs is:  $460^\circ\text{C}$ , 30 sccm  $\text{H}_2$ , 8 sccm  $\text{SiH}_4$ , 2 sccm  $\text{PH}_3$  (1000 ppm in  $\text{H}_2$ ), total chamber pressure is 40 Torr. A typical n-type SiNW growth rate at this condition is  $\sim 0.8\text{--}1.2\text{ }\mu\text{m}/\text{min}$  (*see Note 2*).

7. When the growth is finished, shut down the furnace heating, turn off all the reactant gas lines, and completely open the valve to fully evacuate the quartz tube. When the quartz tube drops to room temperature, close the pumping valve, and start to flow Ar gas to vent the quartz tube, and then take out the chip. The growth chip should now be fully covered by a layer of grown SiNWs, which can be observed by a high-magnification optical microscope (Olympus) under the dark field mode, or by a scanning electron microscope (*see Fig. 1B and Note 3*).

8. It is suggested to store the as-grown SiNWs on growth chips in a desiccator to reduce the degradation rate. Before it is used for device fabrication, gently sonicate the chip in 1-2 ml ethanol for 5-10 sec. The SiNWs are transferred from the growth chip to the ethanol solution (*see Note 4*).

### 3.2. Fabrication of SiNW-FET devices

1. Take a new silicon  $\langle 100 \rangle$  wafer with 600 nm thermal oxide, and use a diamond scribe to cut it into  $2\text{ cm} \times 2\text{ cm}$  sizes. (These chips will be used as sensor chips for fabrication of SiNW FET devices on them, followed by surface chemical functionalization.) Sonicate these chips in acetone and ethanol, 10 min each.

2. Drop-wise deposit the SiNW-containing ethanol solution on the sensor chip using 1  $\mu\text{l}$  pipette, and allow each drop to fully dry before depositing the next drop. Increasing the number of drops increases the SiNW density on the sensor chip surface, and the deposited SiNWs are observed by a high magnification optical microscope (Olympus) under the dark-field mode. A suggested

SiNW density is  $>1$  nanowire per  $100\text{ }\mu\text{m}^2$ , while the optimal nanowire density should depend on the corresponding electrode pattern design (*see Note 5*).

3. Sequentially, spin-coat two layers of polymer resists on the sensor chip surface. First, place the chip on a spin coater, and cover the chip surface with 0.5 ml LOR3A. Then, spin the chip at 300 g for 40 sec, followed by baking the chip on a hot plate at  $185^{\circ}\text{C}$  for 5 min. Second, place the chip on the spin coater again, and cover its surface with 0.5 ml S1805, then, spin the chip at 300 g for another 40 sec, followed by baking the chip on a hot plate at  $115^{\circ}\text{C}$  for 90 sec.

4. Photolithography step: Place the chip on a photolithography mask aligner, and put a photomask with designed electrode patterns on top of the chip. Illuminate both the photomask and the chip under 365-430 nm UV light for 0.8 sec (*see Note 6*).

5. Gently rinse the UV-illuminated chip in a cup of MF319 developer ( $\sim 50$  ml) for 1 min, and then rinse the chip in 50 ml DI water for another min. Dry the chip surface by a  $\text{N}_2$  gas stream.

6. Use oxygen plasma to clean the sensor chip: 30 W plasma power, 50 sccm  $\text{O}_2$  flow, for 60 sec.

7. Immerse the sensor chip into a cup of buffer hydrofluoric acid (HF) solution for 5-8 sec to etch the native oxide from the SiNW surface, then in 30-50 ml DI water for 10 sec. Quickly ( $<10$  sec) dry the chip by a  $\text{N}_2$  gas stream. Then, immediately place the chip into a thermal metal evaporator and start to evacuate the evaporator chamber (*see Note 7*).

8. When the base pressure of the evaporator chamber drops to below  $10^{-6}$  Torr range, turn on the high voltage of the thermal evaporator to evaporate the metal source onto the chip surface with a typical evaporation rate of 0.1 nm/s.

9. When the metal evaporator is finished (e.g.,  $\sim 60$  nm nickel), turn off the high voltage, and wait a few minutes until the samples cool down, and then, vent the chamber and take out the

sensor chip. Then, immediately transfer the chip into a plasma-enhanced chemical vapor deposition (PECVD) chamber.

10. Use the following condition to deposit  $\text{Si}_3\text{N}_4$  onto the sensor chip surface: 20 sccm Ar, 40 sccm  $\text{SiH}_4$  (3%), 6 sccm  $\text{N}_2$ , 5 mTorr total chamber pressure, 375 W microwave power, and a deposition time of 10 min. The  $\text{Si}_3\text{N}_4$  thickness is  $\sim 20\text{-}30$  nm.

11. Place the sensor chip into 30 ml Remover PG solution at  $70^\circ\text{C}$  for 30 min to fully dissolve off the remaining polymer resist, then rinse with acetone and ethanol for 10 sec each, and dry in a  $\text{N}_2$  gas stream.

12. Place the sensor chip into a rapid thermal processor, and anneal the chip at  $380^\circ\text{C}$  for 2 min in a forming gas (10%  $\text{H}_2$ /90%  $\text{N}_2$ ). Low resistance of NiSi will be formed at the interface between SiNWs and Ni electrode contact positions (*see Note 8*).

13. To test the quality of the SiNW FET devices, a current-voltage ( $I$ - $V$ ) test can be carried out in a probe station with an electrical measurement setup (including a lock-in amplifier, a current pre-amplifier, a multifunctional DAC card, and an I/O board). The electrical connection of these instruments and the SiNW FET chip is schematically shown (*see Fig. 2A*). Typically, (i.) current ( $I_{ds}$ ) versus bias voltage ( $V_{ds}$ ) for several gate voltages ( $V_{gs}$ ) and (ii.)  $I_{ds}$  versus  $V_{gs}$  for  $V_{ds} = 1$  V are measured. A set of representative data for p-type and n-type SiNW devices are shown (*see Fig. 2B-E and Note 9*).

### **3.3. Surface modification of SiNW biosensors with monoclonal antibody**

1. Use oxygen plasma to clean the sensor chip: 30 W plasma power, 50 sccm  $\text{O}_2$  flow, for 60 sec. The plasma-cleaner chip is used promptly for surface chemical functionalization.

2. Prepare fresh 1% (v/v) 3-(trimethoxysilyl)propyl aldehyde in ethanol/H<sub>2</sub>O (95%/4%, v/v), and let it sit for 2 min before filtering the solution with a 0.2 µm cut-off syringe filter. Then, immerse the plasma-cleaned sensor chip in the filtered solution for 30 min.
3. Rinse the sensor chip with copious of ethanol for ~ 1 min, and dry in a N<sub>2</sub> gas stream. Then place the chip in an oven, and bake at 110°C for 10 min. Afterwards, use the silanized chip promptly.
4. Mount the sensor chip on a chip carrier using a double-sided conductive carbon tape. Carefully place a single channel microfluidic PDMS stamp on top of the sensor chip so that the channel region overlaps the SiNW FET region well. Then clamp the PDMS stamp onto the chip carrier, and plug in two polyethylene tubes through the PDMS microfluidic channel inlet and outlet holes (*see Note 10*).
5. Prepare a fresh solution of 10-100 µg/ml monoclonal antibodies to prostate specific antigen in the antibody binding buffer. Use a syringe pump to slowly inject the solution through the polyethylene tubes onto the chip surface, with an inject speed ~ 0.2 ml/h. Allow reacting for 2-4 h at room temperature (*see Figure 3 and Note 11*).
6. Use a new syringe to inject a freshly prepared solution of 100 mM ethanolamine in the antibody binding buffer, and allow reacting for 1-2 h to passivate the unreacted aldehyde groups on SiNW surface.
7. Rinse the surface by injecting the antibody binding buffer for 10 min. The sensor chip is now ready for protein detection experiments.

### **3.4. Protein detection by SiNW biosensors**

1. Dilute a known PSA stock solution into different concentrations, typically from high nanomolar (nM) to low picomolar (pM) in the sensing buffer. Store the prepared antigen

solutions in ice before use. Also, use the same sensing buffer to dilute the unknown sample of PSA (*see* **Note 12**).

2. Place the chip carrier, with sensor chip and PDMS stamp, on a probe station stage, and use the microscope and two probe arms of the probe station to select the individual SiNW FET devices. If a second electrical measurement setup (including a lock-in amplifier, a current pre-amplifier, a multifunctional DAC card, and I/O board) is available, two SiNW devices in the same sensor chip can be simultaneously measured. An alternating current (AC) voltage (30-50 mV, 79 Hz) is then applied to measure the device's electrical conductivity. Under the suggested doping level of SiNW devices, conductivity of several hundred to several thousand nanosiemens (nS) is generally suitable for the sensing experiment. This is regarded as the baseline for the selected SiNW sensor.

3. Use the syringe pump to sequentially inject different concentrations of PSA solution onto the device surface, with an injection speed  $\sim 0.2$  ml/h. Keep recording the SiNW sensor conductivity versus the time plot during this process. When PSA binds to its specific antibody on the SiNW sensor surface, an increase of SiNW electrical conductivity is observed for a p-type SiNW device, and the buffer pH is greater than the pI of PSA. The magnitude of conductivity change from the baseline is regarded as the signal induced by the specific binding at this PSA concentration (*see* upper curve in **Fig. 4A**). If the measured SiNW is not modified with antibodies specific to the target antigen or only passivated with ethanolamine, no observable conductance signal can be recorded (*see* lower curve in **Fig. 4A**).

4. When one p-type and one n-type SiNWs in a same chip are simultaneously measured, signals with similar time scale are observed from both SiNW sensor devices. Moreover, these signals will show opposite signs of conductance change. For example, in the case of PSA detection, the

binding of negatively charged PSA will cause the increase and decrease of conductivity of the p-type and n-type SiNW elements, respectively, as shown (*see Fig. 4B*). Therefore, these complementary electrical signals from p-type and n-type SiNW sensors provide a simple, yet robust means for detecting false-positive signals from either electrical noise or nonspecific protein binding to one device (i.e., real and selective binding events must show complementary responses in the p-type and n-type devices).

5. Changing the injection, from the PSA solution to the pure sensing buffer, leads to the unbinding of PSA from the SiNW sensor surface and brings the sensor's conductivity back to its baseline. Use different syringes for different solutions.

6. After all the known PSA solutions are injected and measured, plot the signal versus PSA concentrations to get a calibrated curve for this SiNW sensor device.

7. Inject a solution with unknown PSA concentration onto the sensor chip surface, and record the electrical signal change over this process. Repeat this step for another 2 x to get an average value of signals. Then use the calibrated plot to find the corresponding concentration for this unknown PSA sample.

### **3.5. Multiplexed antibody functionalization and cancer marker detection by SiNW biosensors**

1. Three cancer marker proteins, PSA, CEA, and mucin-1, are selected for demonstration of multiplexed electrical detection by nanowire biosensors. The SiNW device chip is first treated with oxygen plasma followed by 3-(trimethoxysilyl)propyl aldehyde (*see Subheading 3.3. items 1-3*).

2. Prepare 100  $\mu\text{g/ml}$  fresh monoclonal antibody solutions to PSA, CEA, and mucin-1, respectively. Then use a pipette to deposit a few droplets of each antibody solution onto different areas of the SiNW FET array, as schematically shown (*see* **Fig. 5A** and **Note 13**).
3. Allow the sensor chip to sit at room temperature for 3-4 h. Continue to add some more droplets to the same device area to avoid the antibody solution from drying on the chip surface.
4. Mount the chip onto a chip carrier and attach it to a PDMS microfluidic channel (*see* **Subheading 3.3., item 4**).
5. Passivate the chip surface with ethanolamine (*see* **Subheading 3.3., item 6**). Afterward, inject the antibody binding buffer followed by the sensing buffer (*see* **Subheading 3.3., item 7**).
6. Prepare different concentrations of PSA, CEA, and mucin-1 solutions by diluting the stock solutions with the sensing buffer. Store the prepared antigen solutions in ice before use.
7. Use three measurement setups, including the lock-in amplifier, the current pre-amplifier, a multifunctional DAC card and I/O board, to electrically connect three SiNW sensor devices, each pre-incubated with different antibody droplets. Select 17 Hz, 37 Hz, and 79 Hz in the three lock-in amplifiers to apply AC inputs to the corresponding SiNW devices. Follow the measurement and observation as described (*see* **Subheading 3.4., items 3 to 5**). The binding of a type of cancer marker will lead to the conductance change of the corresponding SiNW device as shown (*see* **Fig. 5B**).
8. After each injection of a cancer marker solution, inject the pure sensing buffer to unbind the antigens from the SiNW sensor surface, and bring the sensors' conductivity back to their baselines. Use different syringes for different solution injections. Then acquire the concentration dependence of each measured cancer marker protein.

#### **4. Notes**

1. Store gold nanoparticle colloid in 0-4°C refrigerator, and dilute and use fresh just before deposition onto the growth chip. The deposition time should be kept within 10 sec to avoid gold nanoparticle aggregation, which will produce SiNWs with a much broader diameter distribution.
2. SiH<sub>4</sub>, B<sub>2</sub>H<sub>6</sub>, and PH<sub>3</sub> are toxic and flammable. Use with extreme caution and avoid leakage into air.
3. If the as-grown SiNWs have significantly non-uniform diameters, it is probably due to the aggregation of gold nanoparticle catalysts (*see Note 1*) or to significant radial growth that is simultaneous with axial elongation. This non-uniform diameter distribution should be corrected; otherwise, it will lead to non-uniform and irreproducible electrical properties of SiNW devices. To correct the radial growth case, reduce the CVD growth temperature and/or the total growth pressure until a uniform diameter distribution is observed.
4. Note that SiNWs, stored in a dried N<sub>2</sub> atmosphere, will maintain their quality for at least 1 month. After transferring to ethanol solution, SiNWs can be saved for 1-2 weeks but do degrade with time, especially when there is a small amount of water in ethanol or if the air moisture is high. In addition, if the SiNW solution contains substantial particles or short broken nanowires, reduce the sonication power and time.
5. In order to achieve a good yield of reproducible SiNW FET devices, it is important to disperse SiNWs uniformly in the electrode array region at a density that matches to the metal electrode size and spacing. If deposited SiNWs are significantly aggregated over some area of the chip, the most possible cause is that the chip is not cleaned well or the SiNW-ethanol solution contains a small amount of water.



6. The photoresist exposure time on a mask aligner depends on the power of UV light, which varies with a different mask aligner model and setup. Therefore, it is necessary to run a few tests to get a suitable exposure time.
7. Removing the silicon oxide layer, from the SiNW surface by HF acid, is important for obtaining a good SiNW-metal electrode contact, but do not etch longer than necessary (i.e., ~ 5-10 sec). Use the HF acid with extreme caution and protection, as its solution and vapor are highly corrosive to eyes and skin. Once the chip is etched in HF and rinsed in DI water, immediately place it into the thermal evaporator and begin pumping down the chamber, as the silicon oxide layer will regrow in air quickly.
8. Note that SiNW FET device chips can be stored in a dried N<sub>2</sub> atmosphere for a few days, but use within a week.
9. If the SiNW electrical measurement shows a low current ( $I_{ds}$ ) level and/or non-linear  $I_{ds}$  versus bias voltage ( $V_{ds}$ ), the most common reason is due to poor electrical contact. In this case, repeat the sensor chip fabrication procedure, and pay careful attention to the HF etching and metal deposition steps. Also, the lift-off and subsequent annealing steps should be carried out promptly right after the metal deposition.
10. Overlap the sensor device region with the PDMS fluidic channel well, without making direct contact with the PDMS surface. Otherwise, the surface modification layer will be contaminated.
11. It is important not to use antibody solutions that contain bovine serum albumin (BSA) as a preservative, as it will block the SiNW sensor surface. In addition, azide salts, if present, must be removed from the antibody solution before use.
12. The sensing buffer is prepared with a low concentration of salt to reduce the charge screening effect on the SiNW sensor surface, as the electrolyte double layer thickness is inversely

proportional to the square root of the ionic strength of a buffer. In addition, it is important to choose a suitable pH for the sensing buffer. The SiNW sensor electrical signal is expected to relate with the antigen's surface charge, which indicates that the buffer's pH should be away from the pI of a specific antigen analyte without affecting its activity. Therefore, a pH value of pI  $\pm 2$  is suggested for preparing the buffer.

13. Be careful not to let the antibody droplets dry or to mix different types of antibody droplets. Adding 2-5% glycerol into the antibody solutions can effectively reduce the solvent evaporation rate.

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## Figure Captions

### **Fig. 1: Synthesis of semiconductor nanowires.**

(A) Scheme of a CVD setup for SiNW synthesis. (B) Picture of the center portion of a CVD furnace, where a growth wafer (circled) is sitting inside a quartz tube. Inset: Scanning electron microscopy image of the as-grown SiNWs.

### **Fig. 2: Nanowire FET electrical measurement.**

(A) Schematic of an electrical measurement setup, in which the device chip with SiNWs is connected with a lock-in amplifier, a current pre-amplifier, and a multifunction I/O & NI-DAQ data acquisition card. (B) Family data curve of current ( $I_{ds}$ ) versus bias voltage ( $V_{ds}$ ) plots at different gate voltages for a typical p-type SiNW FET. The red, orange, green, cyan, blue, magenta, red, green, and magenta curves (follow the direction of the arrow) correspond to gate voltage ( $V_g$ ) values of  $-5$ ,  $-4$ ,  $-3$ ,  $-2$ ,  $-1$ ,  $0$ ,  $1$ ,  $2$ , and  $3$  V, respectively. (C)  $I_{ds}$  versus  $V_g$  recorded for the same p-type SiNW FET device in B plotted on linear (blue) and logarithmic (red) scales at a  $V_{ds}$  of  $1$  V. (D) Family data curve of current ( $I_{ds}$ ) versus bias voltage ( $V_{ds}$ ) plots at different gate voltages for a typical n-type SiNW FET. Different  $V_g$  values ( $-5$ ,  $-4$ ,  $-3$ ,  $-2$ ,  $-1$ ,  $0$ ,  $1$ ,  $2$ , and  $3$  V) were plotted using the same color code as in d. (E)  $I_{ds}$  versus  $V_g$  recorded for the same n-type SiNW FET device in D plotted on linear (blue) and logarithmic (red) scales at a  $V_{ds}$  of  $1$  V.

**Fig. 3: Schematic of antibody modification at SiNW surface, including: (1) aldehyde-silane modification; (2) antibody modification; (3) silane passivation by ethanolamine and (4) sensing measurement.**

### **Fig. 4: Real-time SiNW electrical sensing results.**

(A) Conductance versus time data recorded simultaneously from two p-type SiNW devices in an array, whereby, NW1 is functionalized with PSA antibody, and NW2 is modified with

ethanolamine. The vertical lines correspond to times when solutions of (1) 9 pg/ml PSA, (2) 1 pg/ml PSA, (3) 10  $\mu$ g/ml BSA, (4) a mixture of 1 ng/ml PSA and 10  $\mu$ g/ml PSA antibody are delivered to the microfluidic channel. Black arrows in A and B indicate a switch of solution flow from PSA solution to pure buffer. (B) Complementary electrical sensing of PSA using a p-type (NW1) and a n n-type (NW2) SiNW devices. Vertical lines correspond to addition of PSA solutions of (1,2) 0.9 ng/ml, (3) 9 pg/ml, (4) 0.9 pg/ml, and (5) 5 ng/ml. Arrows on the bottom represent the injections of sensing buffer solution.

**Fig. 5: Multiplexed nanowire functionalization and multiplexed electrical detection cancer marker proteins.**

(A) Schematic illustrating multiplexed protein detection by a three SiNW devices in an array. Devices 1, 2, and 3 are fabricated from the similar SiNW, and then, differentiated with distinct monoclonal antibody receptors (1, red; 2, green; 3, blue) specific to three different cancer markers. (B) Conductance vs. time data recorded for the simultaneous detection of PSA, CEA, and mucin-1 on p-type SiNW array in which NW1, NW2, and NW3 are functionalized with monoclonal antibodies for PSA, CEA, and mucin-1, respectively. The solutions are delivered to the SiNW array sequentially as follows: (1) 0.9 ng/ml PSA, (2) 1.4 pg/ml PSA, (3) 0.2 ng/ml CEA, (4) 2 pg/ml CEA, (5) 0.5 ng/ml mucin-1, (6) 5 pg/ml mucin-1. Buffer solutions are injected following each protein solution at points indicated by black arrows.

**Figures 1-3 Legend - Permission**

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**Figure 4 – Legend -Permission**

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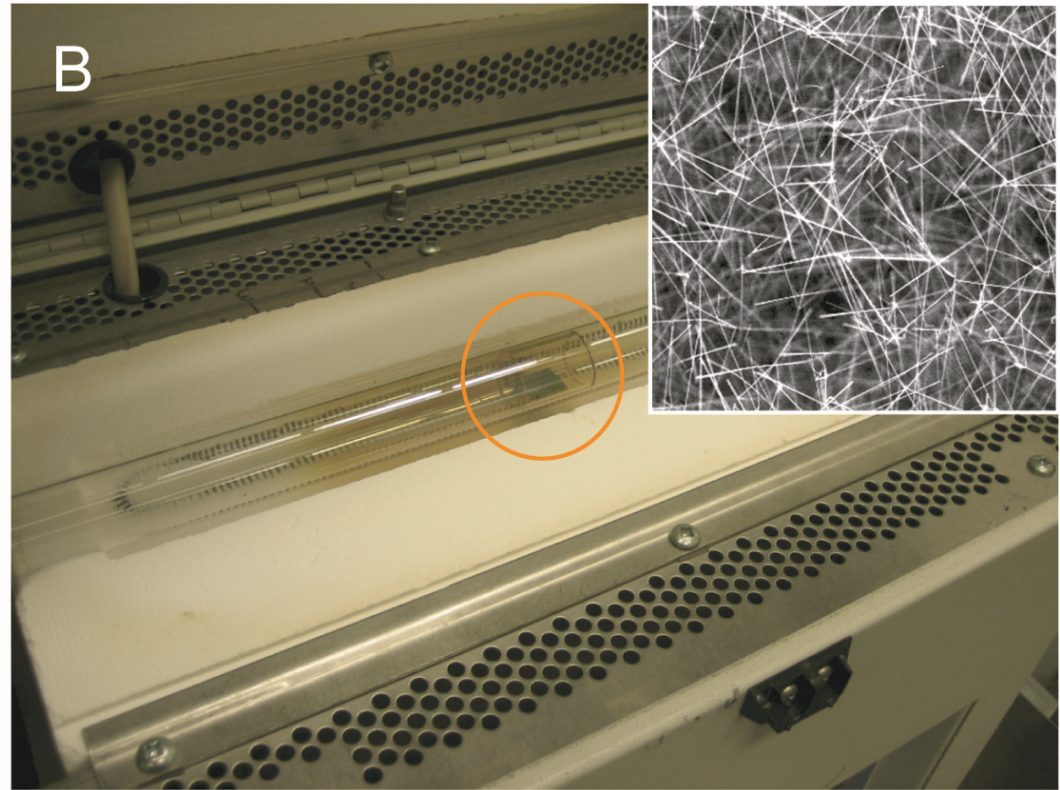
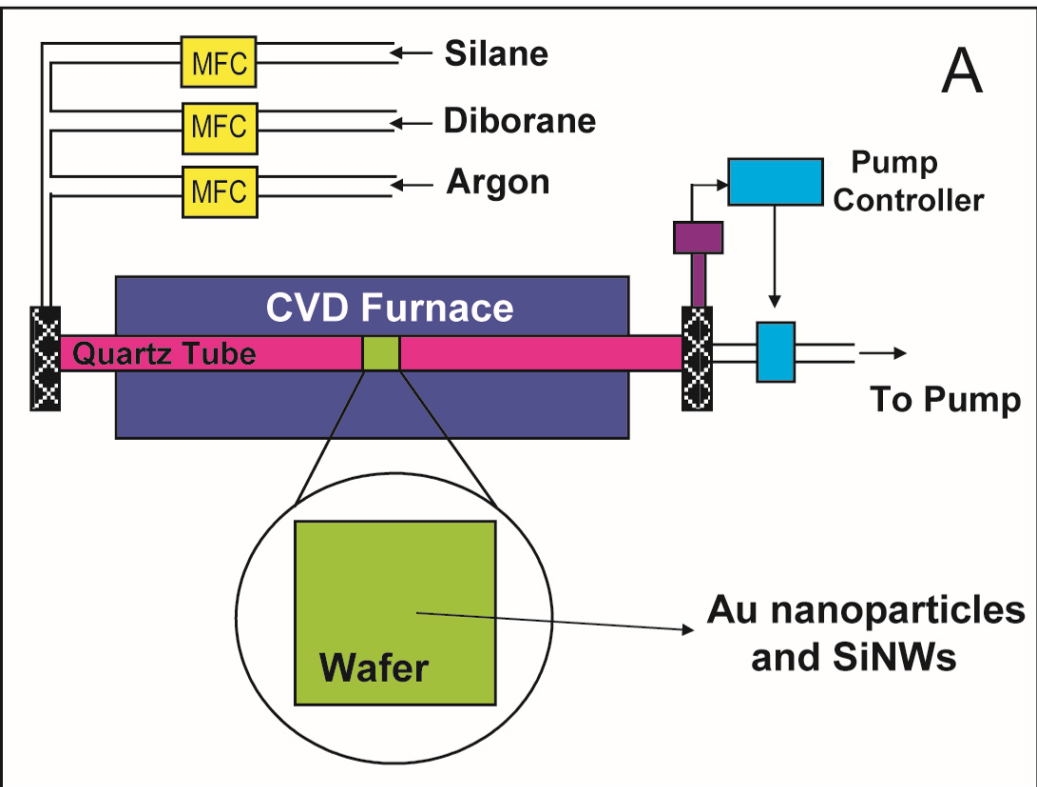
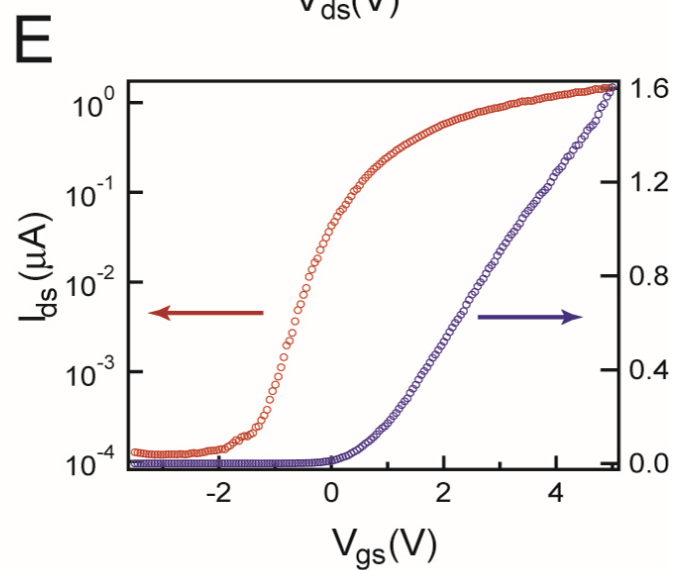
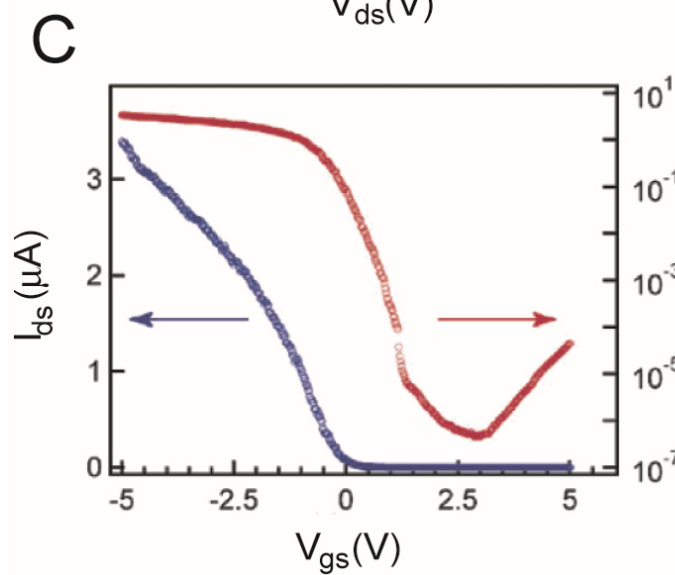
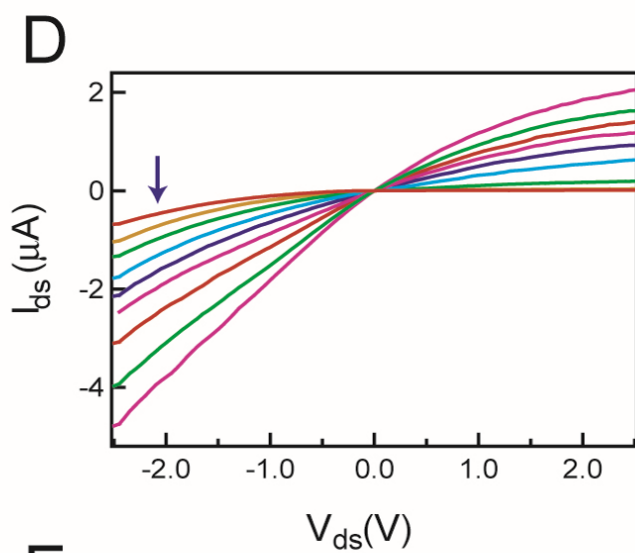
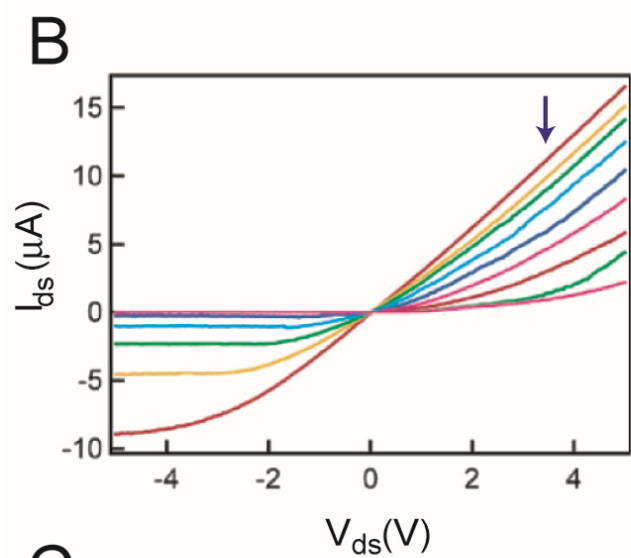
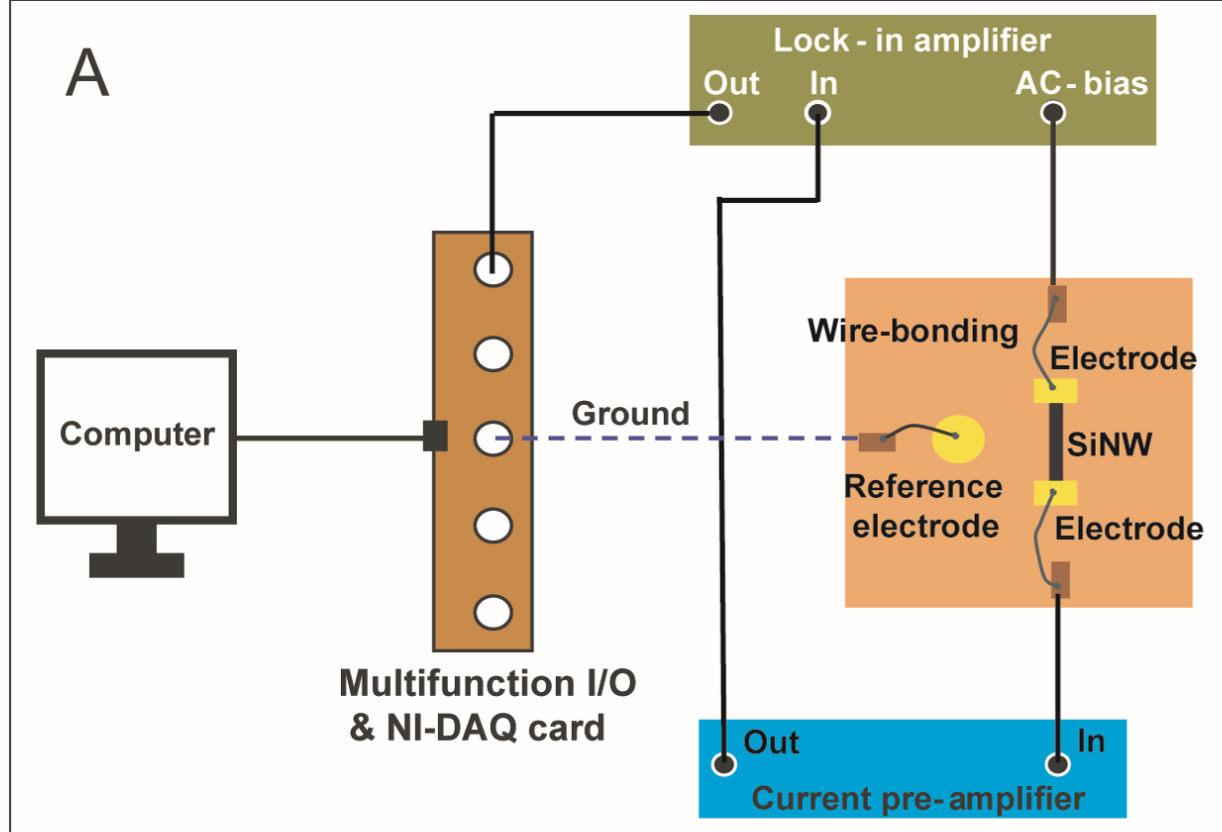


Fig. 1



**Fig. 2**



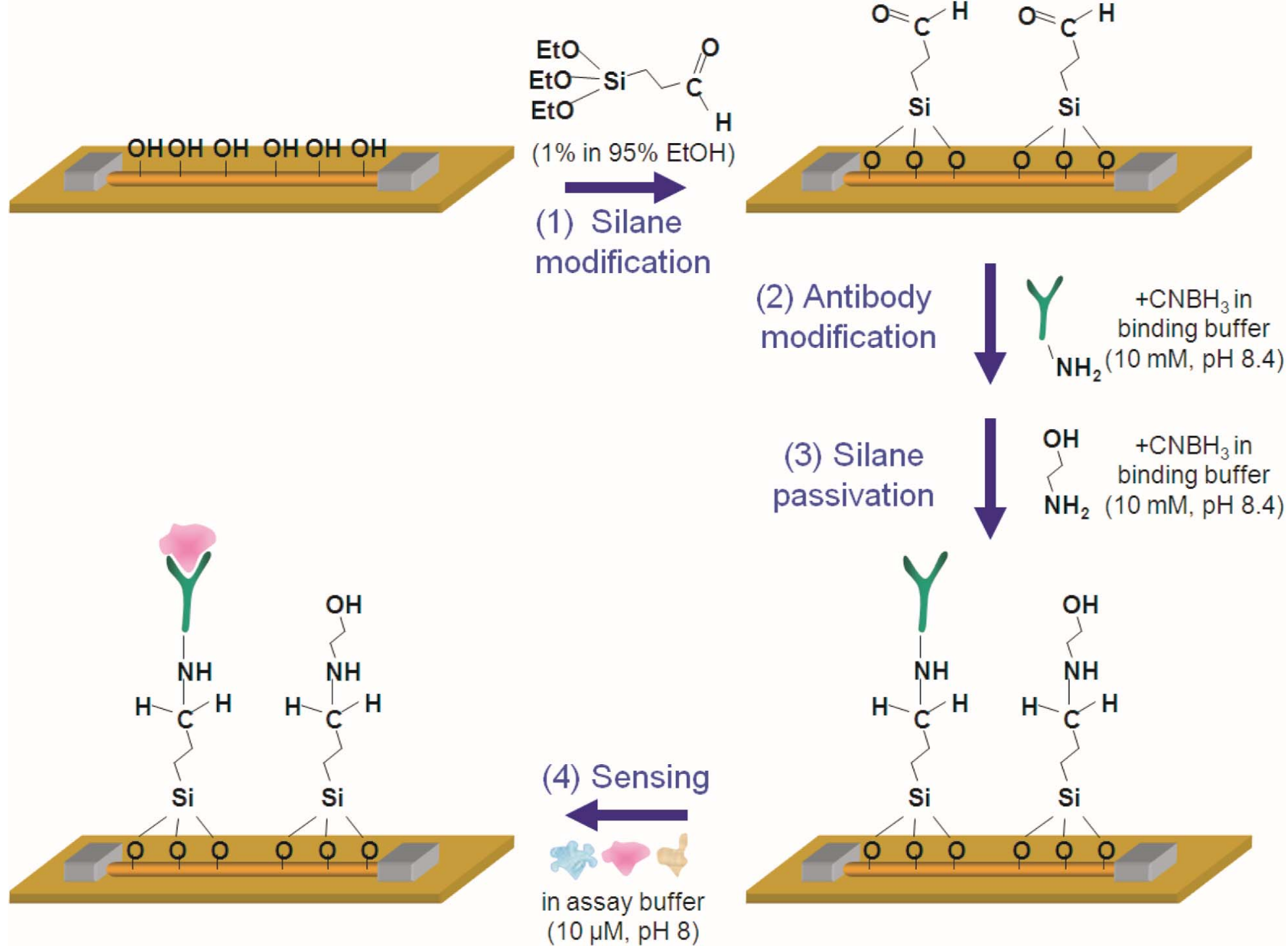
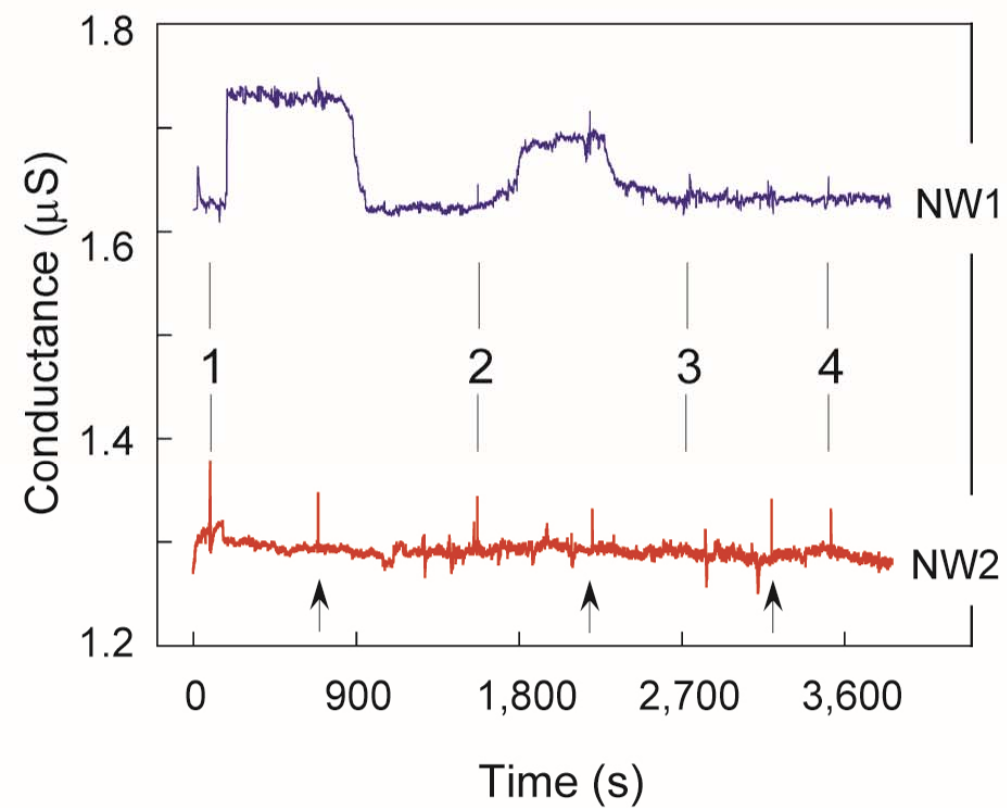
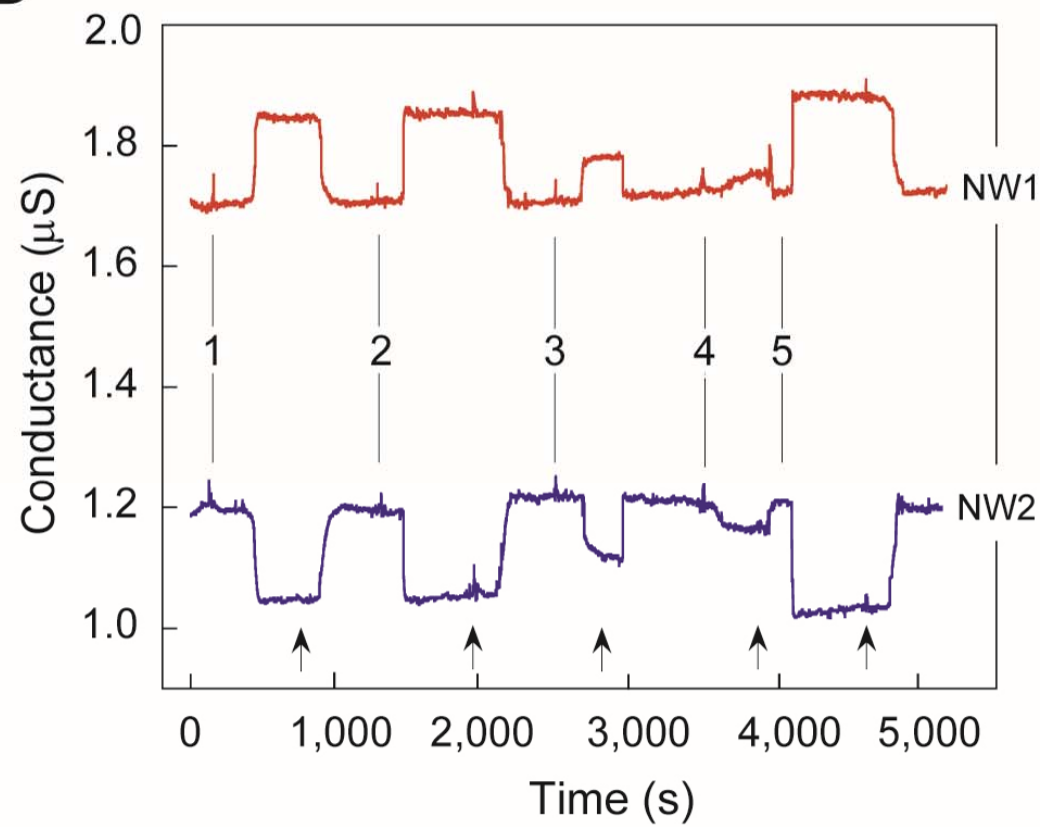


Fig. 3

**A****B****Fig. 4**

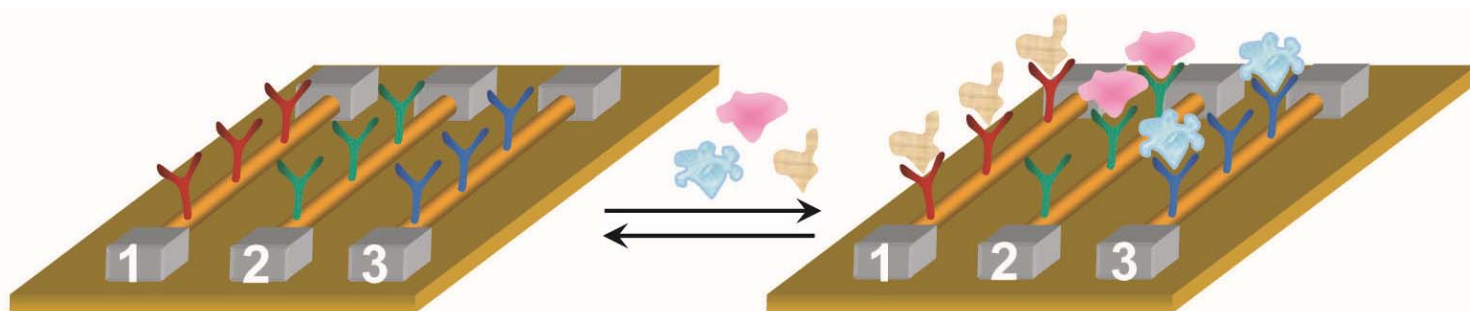
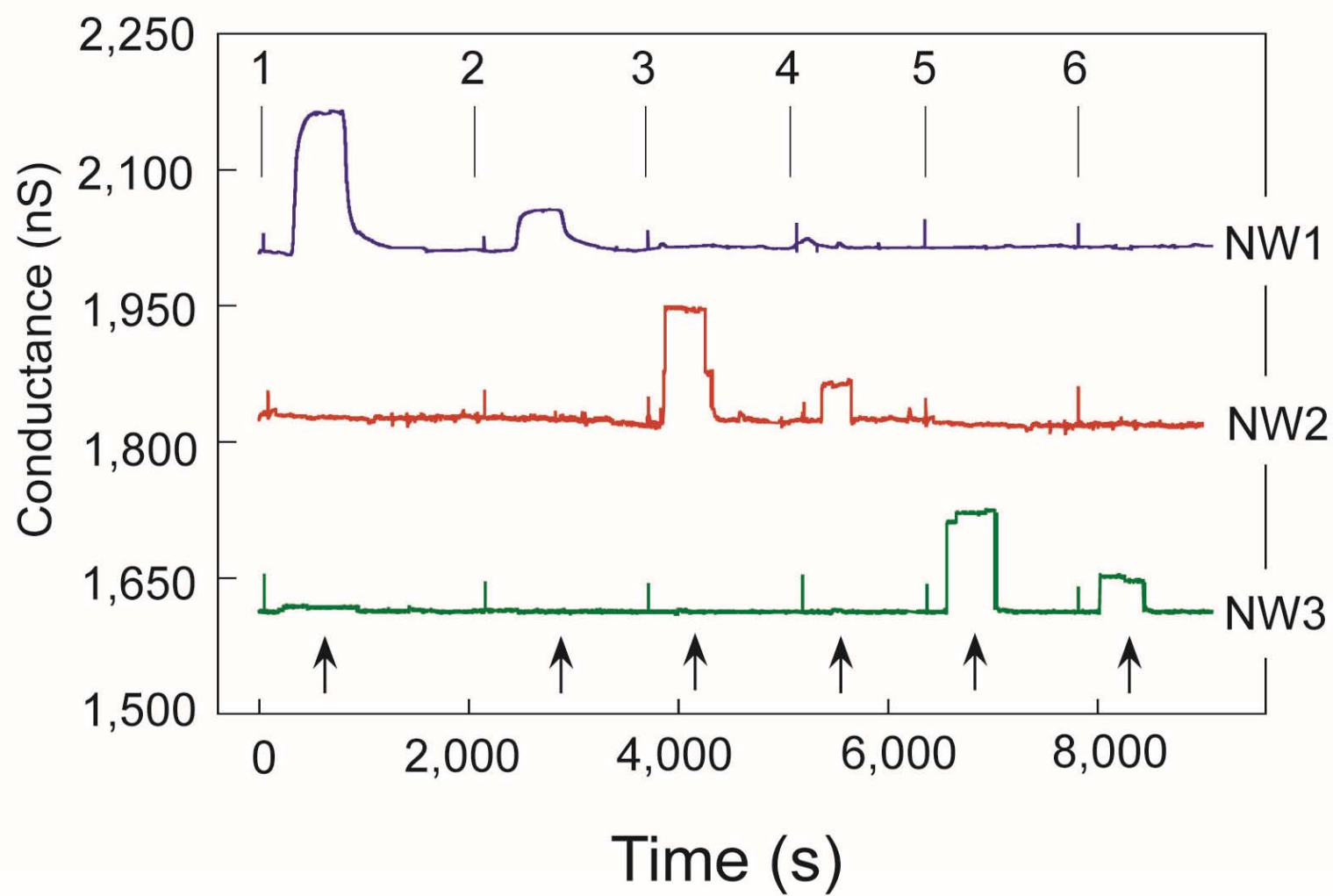
**A****B**

Fig. 5